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MITOTIC ACTIVITY OF HAIRLESS MOUSE EPIDERMAL MELANOCYTES: ITS ROLE IN THE INCREASE OF MELANOCYTES DURING ULTRAVIOLET RADIATION*

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ABSTRACT

Using split epidermal sheets treated with colchicine, a quantitative study was carried out on hairless mouse epidermal melanocytes and those in mitosis, during 14 daily exposures to UV. It was demonstrated that the mitotic figures were first noted after 3 days of irradiation and their incidence varied with the duration of UV treatment; the number of cells in arrested mitosis during the 3 hour period was 0.2–0.4, 0.6–0.7 and 1.0–1.3 per cent of dopa-positive melanocytes, respectively, after 3, 5 and 7 to 14 days of exposure. Comparison of those values and the rate of population increase of melanocytes indicates that the numerical increase in melanocytes during the first 5 days is attributable largely to a mechanism other than the division of melanocytes. In addition, it is likely that the population increase between the 5th to 14th days is due, to a considerable extent, to a similar process. In regard to the mechanism accounting for the population increase in melanocytes, the following processes or possibilities, other than the division of melanocytes are discussed: the activation of pigment formation in amelanogenic melanocytes and the migration of dermal melanocytes into the epidermis.

It is generally accepted that repeated irradiation with ultraviolet light (UV) induces a population increase of melanocytes in the epidermis of man and experimental animals. In regard to the mechanism of this phenomenon, the following processes or possibilities have been considered: a) the division of melanocytes; b) the activation of pigment formation in amelanogenic melanocytes; c) the migration of dermal melanocytes into the epidermis; d) various combinations of these processes (1). The division of melanocytes has been shown in this phenomenon in murine skin by Quevedo *et al.* (2). Further, published reports have provided evidence to support the occurrence of activation of amelanogenic melanocytes in man (3) and experimental animals (4–7). However, the relative contribution of each process remains to be established. In our preceding study using tritiated thymidine, it was demonstrated that approximately 1.1 per cent of dopa-positive epidermal melanocytes were labeled in hairless mice receiving 9 daily exposure to UV (8). However, an attempt to trace the labeled melanocytes using split epidermal sheets was unsuccessful. In the present study, therefore, using colchicine-treated epidermal sheets, we have attempted to determine, quantitatively, epidermal melanocytes in mitosis at several intervals during UV exposures in hairless mice.

MATERIALS AND METHODS

The hairless mice used in this study were of the strain used in previous studies (6–9). Male animals weighing from 16 to 24 gm were studied. An area 1 × 3 cm^2 on the back of each animal was irradiated with a germicidal lamp, especially designed by Mitsubishi Electric Co. Approximately 97 per cent of the emission energy of the lamp is at 2536.5 Å. The lamp-to-target distance was 10 cm; the time of exposure was 5 minutes. Each exposure delivered 19.5 × 10^5 erg/cm^2 of energy. This was done at 11:00 a.m. to noon daily, up to a total of 14 exposures. Three animals on each day were sacrificed, between 11:00 a.m. and noon, 24 hours after 1, 3, 5, 7 and 14 days of UV treatment. Those animals which were killed did not receive irradiation on the day of death. Three hours prior to sacrifice, colchicine of 0.05 mg per 20 gm of body weight (Lilly & Co.) was injected subcutaneously.

Skin specimens were taken from the irradiated sites immediately after killing. Control specimens were obtained from the backs of three nonirradiated animals treated with colchicine prior to sacrifice. Epidermal sheets were prepared by incubating the skin specimens in 2N sodium bromide solution for 2–3 hours at room temperature. The isolated epidermal sheets were placed in 6 per cent neutral formalin for 1 hour at 4° C and then incubated in 0.1 per cent dopa in 0.1M phosphate buffer, pH 7.4, for 1 hour at 37° C. These sections were counterstained with Mayer's hematoxylin and mounted.

Cell counts were carried out on dopa-positive melanocytes and those in mitosis, at random, in the epidermal sheets using the oil immersion lens. The melanocyte population was expressed as number of cells per square mm of skin surface according to Staricco and Pinkus (10).

RESULTS

Numbers of dopa-positive melanocytes and those in mitosis assessed at various intervals are summarized in Table I. Quantitation of the dividing melanocytes following mitotic stages was not performed because it was occasionally difficult to ascertain distinctly each stage of mitosis in these cells using the epidermal sheets prepared

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TABLE I

Quantitative assessments of dopa-positive melanocytes in division during daily exposure to UV

Animal no.	Body weight gm	Days of irradiation	Total number of cells counted (A)	Number of cells in division (B)	B/A per cent
1	22	Control	42	0	
2	22	Control	48	0	
3	17	Control	52	0	
4	20	1	75	0	
5	20	1	48	0	
6	20	1	135	0	
7	20	3	1376	6	0.4
8	21	3	1635	5	0.3
9	22	3	925	2	0.2
10	22	5	1688	10	0.6
11	22	5	3394	24	0.7
12	24	5	3981	26	0.6
13	19	7	2036	20	1.0
14	16	7	3795	49	1.3
15	16	7	3592	43	1.2
16	22	14	9433	94	1.0
17	21	14	9824	105	1.1
18	21	14	9780	98	1.0

in this study. The Figure shows mitotic figures of the melanocytes. The cells in mitosis had a tendency to retract the dendritic processes and also to possess larger cell bodies than those in interphase (Fig., secs. A, B, C), however, in some instances the dendrites were well preserved (Fig. sec. D). Mitotic figures were not detected in control specimens nor in those after a single irradiation. The incidence of mitosis was 0.2–0.4 per cent of dopa-positive melanocytes after 3 days of irradiation and then increased to 0.6–0.7, 1.0–1.3 and 1.0–1.1 per cent, respectively, after 5, 7 and 14 days of treatment.

Average numbers of dopa-positive melanocytes per square mm at the same intervals are summarized in Table II. The cell populations after 2 to 6 weeks of irradiation, which were described in a previous report (7), are listed in this table for comparison. The cell populations were 61 ± 3, 155 ± 3, 190 ± 6 and 407 ± 8 cells/mm^2, respectively, after 3, 5, 7 and 14 days of UV treatment. The number of cells in controls and after a single exposure of UV were too small to be expressed as population per square mm.

DISCUSSION

In spite of the fact that epidermal melanocytes incorporate tritiated thymidine (2, 8, 11), their mitotic figures have rarely been observed in man and certain mammals (12–16). The present study, therefore, was carried out using split epidermal sheets which were treated with colchicine of 0.05 mg per 20 gm of body weight. This study demonstrated that mitotic figures were first detected after 3 days of UV exposure and their incidence varied with the duration of daily treatment as follows: 0.2–0.4, 0.6–0.7 and 1.0–1.3 per cent of dopa-positive melanocytes, respectively, after 3, 5 and 7 to 14 days of treatment. It was confirmed that the dendritic processes of melanocytes, in some instances, were well preserved during mitosis as described by several authors (13–15). It is noteworthy that the incidence of mitosis between the 7th to 14th exposures roughly coincides with that of labeled melanocytes obtained from a previous autoradiographic study using vertical histologic sections from the back skin receiving 9 daily treatments (8).

Our present and previous studies (6, 7) have demonstrated that dopa-positive epidermal melanocytes increased in number during repeated irradiation; the melanocyte populations were approximately 61, 155, 190, 407 and 509–610 cells/mm^2, respectively, after 3, 5, 7, 14 days and 3 through 6 weeks of treatment as indicated in Table II. If this numerical increase was due entirely to the division of melanocytes, the incidence of their mitosis per day would be expected, as shown in Table II, to be approximately 60, 11, 3 and 1–0.7 per cent of dopa-positive melanocytes, respectively, between the 3rd to 5th days, the 5th to 14th days, the 2nd to 3rd weeks and the 3rd to 6th weeks of UV treatment. On the other hand, if the melanocytes divided without a diurnal variation, at the rate as shown in Table I, the incidence of mitosis per day would be calculated to be 1.6–3.2 (0.2–0.4 × 24/3), 4.2–5.6 (0.6–0.7 × 24/3) and 8.0–10.4 (1.0–1.3 × 24/3) per cent, respectively, after 3, 5 and 7 to 14 days of UV exposure. Comparison of those values indicates that the numerical increase in melanocytes during the first 5 days is incuded largely through the mechanism other than cell division. Further, it is likely that the increase between the 5th to 14th days is due, to a considerable extent, to a similar process. In contrast, it seems not improbable that the increase between the 2nd to 6th weeks may be due to melanocyte division.

In regard to the mechanisms other than cell division accounting for the UV-induced increase in epidermal melanocyte population, there are processes or possibilities as referred to previously, i.e. the activation of pigment formation in amelanogenic melanocytes, the migration of dermal melanocytes etc. In hairless mice, the ATPase-positive, dopa-negative dendritic cells almost exclusively situated at the dermoepidermal junction were considered as representing amelanogenic melanocytes by Wolff and Winkelmann (17) and also by the present authors (6, 7). On the other hand, the α-naphthyl esterase-positive dendritic cells were maintained to be identical with this type of melanocyte by Saito *et al.* (18). At the ul-

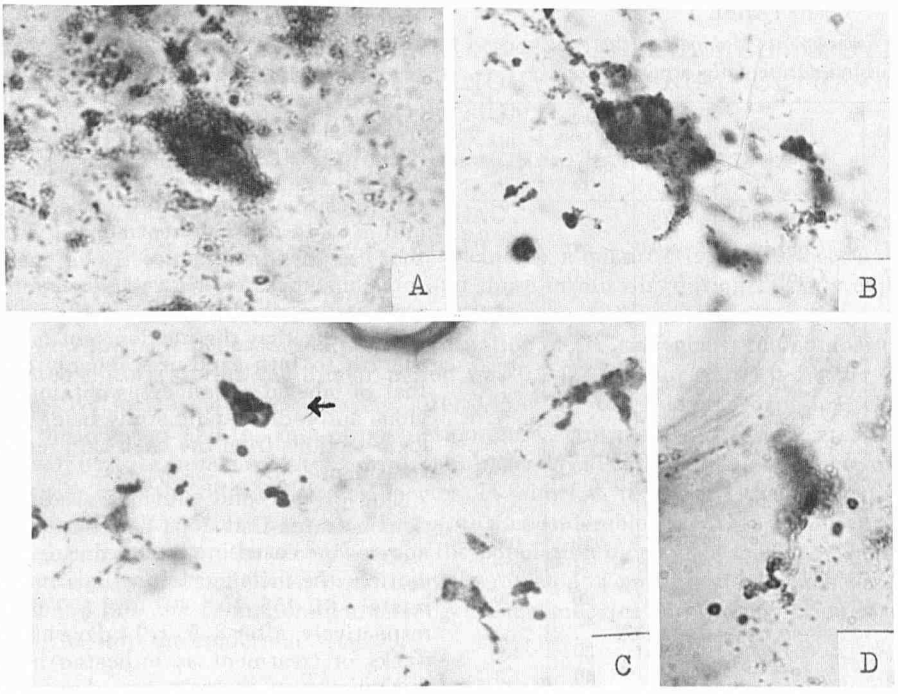

FIGURE. Sections A–D. Mitotic figures of melanocytes. (A, B) Melanocytes showing a tendency to retract the dendrites × 1,500. (C) melanocyte (↑) with larger cell body × 600. (D) cell with a dendritic process × 1,500.

TABLE II

Change in average number of dopa-positive melanocytes per mm² during daily irradiation

Day or week of irradiation	Average number of cells/mm²	Presumed rate of increase in melanocytes per day (per cent)
Control	occasional cells	
1 day	occasional cells	
3 days	61 ± 3	60
5 days	155 ± 3	11
7 days	190 ± 6	11.5
2 weeks	407 ± 8	
2 weeks*	407 ± 52	3
3 weeks*	509 ± 64	1
4 weeks*	546 ± 67	0.9
5 weeks*	583 ± 67	0.7
6 weeks*	610 ± 78	

* From Sato (7). The exposure is daily, 6 times per week.

trastructural level, however, the amelanogenic melanocytes have not yet been identified. Recently a certain type of dendritic cell called an indeterminate dendritic cell has been reported to be present regularly in the epidermis of guinea pigs (19), hairless mice (6, 9, 20) and man (21–23). However, the nature of these cells and their relationship to the melanocytes or Langerhans cells are still controversial. In our previous study using electron microscopy in combination with cytochemical methods for ATPase activity, evidence was not obtained to support a close relationship between the indeterminate dendritic cells and melanocytes in either normal or UV-treated hairless mouse skin (9). On the other hand, dermal melanocytes have been reported to be present in hairless mice (5, 24). In our previous study (7), these cells were found to increase numerically in the upper and middle dermis during repeated irradiation. This numerical increase, however, did not reach significant levels during the first week whereas it became marked after the 3rd week. Thus it seems doubtful that the migration of dermal melanocytes may be involved in the rise in the epidermal melanocyte population during the first week though there is the possibility that the lack of a significant increase in dermal melanocytes during this time may be due to their migration into the epidermis. Although these findings taken together appear to favor the activation of amelanogenic melanocytes accounting for the increase in the epidermal melanocyte population, it has not been completely substantiated.

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